

I. AMENDMENTS

In the specification:

At column 1, please delete the text at lines 4-6 and replace with the following:

-- CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a reissue application of U.S. Serial No. 08/793,408 (U.S. Patent No. 6,007,988), filed June 2, 1997 and issued December 28, 1999 which in turn is a national phase filing of PCT/GB95/01949 pursuant to 35 U.S.C. § 371. More than one reissue application has been filed for the reissue of Patent No. 6,007,988 including a continuation of this reissue application designated U.S. Serial No. 10/309,578, filed December 3, 2002.--

Please amend the paragraph beginning at column 10, line 42 as follows:

--FIG. 2 shows an alignment of the three zinc fingers of a single zinc finger protein (Seq ID No. 2) used in the phage display library;--

Please amend the paragraph beginning at column 11, line 1 as follows:

--FIG. 12 shows an alignment of the amino acid sequence of zinc fingers in a single zinc finger polypeptide (Seq ID No. 18) designed to bind to a particular DNA sequence (a ras oncogene).--

Please amend the paragraph beginning at column 9, line 5 as follows:

--To the best knowledge of the inventors, design of a zinc finger polypeptide and its successful use in modulation of gene expression (as described below) has never previously been demonstrated. This breakthrough presents numerous possibilities. In particular, zinc finger polypeptides could be designed for therapeutic and/or prophylactic use in regulating the expression of disease-associated genes. For example, zinc finger polypeptides could be used to inhibit the expression of foreign genes (e.g., the genes of bacterial or viral pathogens) in man or animals, or to modify the expression of mutated host genes (such as oncogenes).--

Please amend the paragraph beginning at column 12, line 34 has as follows:

-- Colonies were transferred from plates to 200 ml 2xTY/Zn/Tet (2xTY containing 50  $\mu\text{M}$   $\text{Zn}(\text{CH}_3\text{COO})_2$  and 15  $\mu\text{g/ml}$  tetracycline) and grown overnight. Phage were purified from culture supernatant by two rounds of precipitation using 0.2 volumes of 20% PEG/2.5M NaCl containing 50,  $\mu\text{M}$   $\text{Zn}(\text{CH}_3\text{COO})_2$  and resuspended in zinc finger phage buffer (20 mM HEPES pH7.5, 50 mM NaCl, 1 mM  $\text{MgCl}_2$  and 50  $\mu\text{M}$   $\text{Zn}(\text{CH}_3\text{COO})_2$ ). Streptavidin-coated paramagnetic beads (Dyna) were washed in zinc finger phage buffer and blocked for 1 hour at room temperature with the same buffer made up to 6% in fat-free dried milk (Marvel). Selection of phage was over three rounds: in the first round, beads (1 mg) were saturated with biotinylated oligonucleotide (~80 nM) and then washed prior to phage binding, but in the second and third rounds 1.7 nM oligonucleotide and 5  $\mu\text{g}$  poly dGC (Sigma) were added to the beads with the phage. Binding reactions (1.5 ml) for 1 hour at 15°C. were in zinc finger phage buffer made up to 2% in fat-free dried milk (Marvel) and 1% in Tween 20, and typically containing  $5 \times 10^{11}$  phage. Beads were washed 15 times with 1 ml of the same buffer. Phage were eluted by shaking in 0.1M triethylamine [triethylamine] for 5 min and neutralised with an equal volume of 1M Tris pH7.4. Log phase *E. coli* TG1 in 2xTY were infected with eluted phage for 30 min at 37°C. and plated as described above. Phage titres were determined by plating serial dilutions of the infected bacteria. --

Please amend the paragraph beginning at column 19, line 62 as follows:

-- Flexible flat-bottomed 96-well microtitre plates (Falcon) were coated overnight at 4°C. with streptavidin (0.1 mg/ml in 0.1M  $\text{NaHCO}_3$  pH8.6, 0.03%  $\text{NaN}_3$ ). Wells were blocked for one hour with PBS/Zn (PBS, 50  $\mu\text{M}$   $\text{Zn}(\text{CH}_3\text{COO})_2$ ) containing 2% fat-free dried milk (Marvel), washed 3 times with PBS/Zn containing 0.1% Tween, and another 3 times with PBS/Zn. The "bound" strand of each oligonucleotide library was made synthetically and the other strand extended from a 5'-biotinylated universal primer using DNA polymerase I (Klenow fragment). Fill-in reactions were added to wells (0.8 pmole DNA library in each) in PBS/Zn for 15 minutes, then washed once with PBS/Zn containing 0.1% Tween, and once again with PBS/Zn. Overnight bacterial cultures each containing a selected zinc finger phage were grown in 2xTY containing 50 mM  $\text{Zn}(\text{CH}_3\text{COO})_2$  and 15  $\mu\text{g/ml}$  tetracycline at 30°C. Culture supernatants containing phage were diluted tenfold by the addition of PBS/Zn containing 2% fat-free dried milk (Marvel), 1% Tween and 20  $\mu\text{g/ml}$  sonicated salmon sperm DNA. Diluted phage solutions (50  $\mu\text{l}$ ) were applied to wells and binding allowed to proceed for one hour at 20°C. Unbound phage were removed by washing [D] 5 times with PBS/Zn containing 1% Tween, and then 3 times with PBS/Zn. Bound phage were detected as described previously

(Griffiths et al., 1994 EMBO J. [In Press] 13(14):3245-3260), or using HRP-conjugated anti-M13 IgG (Pharmacia), and quantitated using software package SOFT-MAX 2.32 (Molecular Devices Corp). --

Please amend the paragraph beginning at column 21, line 61 as follows:

-- Table 2 summarises frequently observed amino acid-base contacts in interactions of selected zinc fingers with DNA. The given contacts comprise a "syllabic" recognition code for appropriate triplets. Cognate amino acids and their positions in the  $\alpha$ -helix are entered in a matrix relating each base to each position of a triplet. Auxiliary amino acids from position +2 can enhance or modulate specificity of amino acids at position -1 and these are listed as pairs. Ser or Thr at position +6 permit Asp +2 of the following finger (denoted Asp ++2) to specify both G and T indirectly, and the pairs are listed. The specificity of Ser +3 for T and Thr +3 for C may be interchangeable in rare instances while Val +3 appears to be consistently ambiguous. --

Please amend the paragraph beginning at column 29, line 55 as follows:

--Immunofluorescence microscopy of transfected Ba/F3+p190 cells in the absence of IL-3 shows chromatin condensation and nuclear fragmentation into small apoptotic bodies, while the nuclei of Ba/F3+p210 cells remain intact. Northern blots of total cytoplasmic RNA from Ba/F3+p190 cells transiently transfected with the anti-BCR-ABL peptide revealed reduced levels of p190<sup>BCR-ABL</sup> mRNA relative to untransfected cells. By contrast, similarly transfected Ba/F3+p210 cells showed no decrease in the levels of p210<sup>BCR-ABL</sup> mRNA [(FIG. 12)]. The blots were performed as follows: 10 mg of total cytoplasmic RNA, from the cells indicated, was glyoxylated and fractionated in 1.4% agarose gels in 10 mM NaPO<sub>4</sub> buffer, pH 7.0. After electrophoresis the gel was blotted onto HYBOND-N (Amersham), UV-cross linked and hybridized to an <sup>32</sup>P-labelled c-ABL probe. Autoradiography was for 14 h at -70°C. Loading was monitored by reprobing the filters with a mouse [b-acting]  $\beta$ -actin cDNA.--

In the claims:

Please amend claims 3, 19, 23 and 32 by deleting the bracketed material and adding the underlined material as follows: